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THE RELEASE OF VASOPRESSIN BY NICOTINE: FURTHER STUDIES ON ITS SITE OF ACTION

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SUMMARY

1. In cats anaesthetized with chloralose the release of vasopressin in response to nicotine injections was examined. This release was measured by assaying the hormone in samples of venous blood.

2. Nicotine injections were given by three different routes, namely intravertebral, intracarotid and intravenous. The first two represent close arterial routes to the medulla and to the hypothalamus, respectively, the effects of which could be compared to those following intravenous, i.e. systemic, administration.

3. Nicotine was found to increase vasopressin secretion by all three routes of administration. The potency of intracarotid injections was found to be no greater than that of intravenous injections, in sharp contrast to intravertebral injections, which were 4–5 times more potent.

4. In terms of vascular effects, intracarotid and intravenous injections of nicotine were found to increase blood pressure, whereas intravertebral injections of low doses of nicotine were always followed by a fall in blood pressure. Higher doses of intravertebral nicotine produce mixed results, pressor or depressor, in different animals.

5. The vasodepressor effect of intravertebral nicotine was part of a cardiovascular response which included a lowering of total peripheral resistance and of stroke work, whereas the cardiac output, the heart rate and the stroke volume remained essentially unchanged.

6. These results clearly indicate that a medullary area, which has been previously described, is the most sensitive site for the vasopressin releasing action of nicotine and that systemic administration of the drug induces vasopressin secretion by virtue of its action on the medulla, rather than directly on the supraoptic nucleus.

7. The results also indicate that the vasodepressor effect which follows the application of nicotine on the medulla is chiefly due to vasodilator effects on systemic blood vessels, with practically no action on cardiac function. The significance of these results is discussed.

INTRODUCTION

It is well established that nicotine produces an antidiuretic action (Burn, Truelove & Burn, 1945) which is due to the release of vasopressin. The site of this vasopressin releasing action of nicotine has not been subject to controversy until quite recently. Pickford (1939) demonstrated that acetylcholine induces an antidiuretic response in atropinized dogs and that this response is absent in animals with experimental diabetes insipidus. Subsequently Pickford (1947) and Duke, Pickford & Watt (1950) showed that the neurones in the supraoptic nucleus exhibit nicotinic-like receptors. It was later demonstrated that local application of nicotine to the supraoptic nucleus induces vasopressin release, which is evidenced by the increase in the rate of discharge along the supraopticohypophysial tract (Barker, Crayton & Nicoll, 1971). No other central site was ever described from which vasopressin release could be induced by nicotine before the discovery, by Bisset, Feldberg, Guertzenstein & Rocha e Silva (1975) of a sensitive site on the ventral surface of the medulla. Topical application of nicotine to this sensitive site was followed by increased vasopressin secretion and by a fall in arterial blood pressure. This observation was made within the framework of a comprehensive study of the actions of various drugs which, on ventricular injection or topical application to the ventral surface of the medulla, affect blood pressure, heart rate, respiration, blood glucose, or hormone secretion (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973; Bousquet & Guertzenstein, 1973; Edery & Guertzenstein, 1974; Feldberg & Gupta, 1974; Guertzenstein & Silver, 1974; Dey, Feldberg & Wendlandt, 1975; Bisset *et al.* 1975).

The finding of a second site of action for the vasopressin releasing activity of nicotine raises the question of which of the two sites is responsible for vasopressin secretion in response to systemically administered nicotine. In the experiments to be described, nicotine injections were given to chloralose anaesthetized cats by three different routes, namely intravertebral, intracarotid and intravenous. The first two represent close arterial routes to the medulla and to the hypothalamus, respectively, the effects of which could be compared to those obtained following intravenous, i.e. systemic administration.

The results clearly indicate that the medullary site is the most sensitive and that systemic administration of nicotine induces vasopressin secretion

by virtue of its action on the medulla, rather than directly on the supraoptic nucleus or on any other site within the distribution space of the carotid arteries. This paper also deals with the arterial hypotension produced by nicotine on the medulla and analyses it in terms of vascular dynamics.

METHODS

Experiments were performed on twenty-five cats of either sex, weighing between 2·5 and 3·7 kg. Anaesthesia was induced with ethyl chloride and ether, followed by an intravenous injection of chloralose (60 mg/kg). The trachea was cannulated to ensure a clear airway, but artificial respiration was not used except for the implanting of flowprobes around the root of the aorta, in six experiments. The following blood vessels were cannulated: right femoral artery and vein, right external jugular vein, left subclavian and left lingual arteries. The cannula in the subclavian was placed with its tip facing centrally and opposite to the emergence of the vertebral artery; the internal thoracic, the omocervical and the thyrocervical arteries were identified and tied off at their respective points of branching. The cannula in the lingual artery was also placed with its tip facing centrally and opposite to the emergence of the lingual from the external carotid. The capacity of each of these two cannulae (0·05–0·09 ml.) was measured in each experiment. A thermistor was placed in the thoracic oesophagus and deep body temperature monitored and maintained between 36·5 and 37·5° C, by means of a heater placed inside the operating table. Upon completion of the surgical preparation, atropine sulphate (1 mg/kg) was given intravenously and 1 hr was allowed before the experiment was begun.

At the end of every experiment a particulate dye, Cobalt Blue, was injected either into the vertebral or the carotid artery, at the constant rate of 0·05 ml./sec, over a period of 15 sec. At the 15th sec of injection the animal was instantaneously killed by means of a guillotine. Dye distribution was visually determined by post-mortem dissection of the brain. The exact position of the subclavian and lingual cannulae, as well as the effectiveness of the ligatures on the branches of the subclavian were also checked at the end of each experiment. Experiments consisted always of injections of nicotine tartrate, in the constant volume of 0·2 ml., delivered at the constant rate of 0·05 ml./sec. In the first 19 experiments (see below) at least one injection was given through each of the three routes used throughout this work: intravenous, intracarotid and intravertebral. Doses of nicotine were calculated in terms of weight of active base/kg body wt. In fifteen experiments, sixty-eight injections of nicotine were given according to the following pattern.

(1) Intravertebral injections were given in the doses of 2·5, 5, 10, 20 and 50 μg/kg.

(2) Intracarotid and intravenous injections were given in the same doses, but additional injections of 100 μg/kg were given by those routes:

(3) Every dose was replicated 4 times for each route.

(4) Each cat received at least one nicotine injection by each of the three routes and no more than six injections in all.

(5) In each case the following parameters were measured, as described below: mean and pulse arterial pressure, heart rate and vasopressin concentration in plasma.

In four other experiments, blood pressure and heart rate were measured following repeated injections of nicotine by each of the three routes, but no vasopressin measurements were made.

Finally in six additional experiments, intravertebral injections of nicotine were given (doses ranging from 2·5 to 50 μg/kg), while the following parameters of haemodynamic function were measured or calculated: mean and pulse arterial blood

pressure, heart rate, aortic flow (measured), stroke volume, stroke work, and total peripheral resistance (calculated, assuming aortic flow approximates to cardiac output). In these experiments no measurements of plasma vasopressin concentration were made.

Blood pressure was measured by means of a strain gauge transducer (Statham, type P 23 dB), connected to the cannula in the femoral artery. The signal from the transducer was displayed on a galvanometric recorder (Beckman, type RM). The pressure signal was displayed as pulse pressure on 1 channel, as mean pressure on a 2nd channel and used to compute heart rate in a 3rd channel. Aortic flow was determined by means of an electromagnetic flowprobe (Statham, type SP 7515). This was placed around the root of the aorta, through an acutely performed thoracotomy at the level of the 4th intercostal space, on the left side; the thorax was then closed and artificial respiration discontinued. The signal from the aortic flowprobe was fed into an electromagnetic flowmeter (Statham, type SP 2202) and recorded on the galvonometric recorder.

The signals for pulse and mean arterial blood pressure, heart rate and aortic flow were simultaneously recorded on tape, by means of an FM tape recorder (Philips, type Analog-7) and stored for future reference.

Vasopressin assay: blood sampling, extraction and assay. Blood samples (5 ml.) were collected from a femoral vein at a constant rate, over 2 min, starting either 15 min before (control samples – *C*) or 30 sec after (test samples – *T*) nicotine injections. The first sample was collected with simultaneous replacement of an equal volume of saline. For subsequent samples, the red cells and remaining plasma from the previous sample collection was made up to 5 ml. with saline and reinjected simultaneously with the next sample collection.

Plasma was extracted with ethanol, as described by Bisset, Hilton & Poisner (1967), with the modifications described by Errington & Rocha e Silva (1972). Antidiuretic activity in the plasma samples was determined by intravenous injections into ethanol-anaesthetized water-loaded rats, according to the method of Dicker (1953) and Bisset (1962), as modified by Clark & Rocha e Silva (1967). Recovery of vasopressin by the extraction and assay methods here described is 80% and the assay method shows a standard deviation/mean ratio of 0·15.

Statistical analysis. Data referring to vasopressin concentrations in plasma were calculated as test/control ratios and these were log converted. The log transformed data were submitted to a two-way model of analysis of variance, to determine the differences between doses (in the 2·5–50 μg/kg range) and between routes of administration (Snedecor & Cochran, 1967).

RESULTS

Vasopressin secretion. Nicotine was found to produce vasopressin secretion by all the three routes of administration used in these experiments. Table 1 summarizes the results. In the following description, a test/control (*T*/*C*) ratio for plasma vasopressin is taken to mean *no secretion* if it is lower than or approximately equal to *1*, *doubtful or threshold secretion*, if smaller than *2*, and *clear-cut secretion*, if equal to or greater than *2*. Table 1 shows that 2·5 μg/kg nicotine was ineffective by all three routes. Intravertebral injections of 5 μg/kg caused clear-cut secretion in two out of four experiments, whereas 10 μg/kg was effective in three out of four experiments. Higher doses were always effective. In contrast,

TABLE 1. Vasopressin content in μu/ml. of plasma collected before (*C*) and after (*T*) nicotine (μg/kg)
(68 injections of nicotine given to 15 cats)

	2·5 μg/kg			5 μg/kg			10 μg/kg			20 μg/kg			50 μg/kg			100 μg/k.		
	C	*T*	*T*/*C*	*C*	*T*	*T*/*C*	*C*	*T*	*T*/*C*	*C*	*T*	*T*/*C*	*C*	*T*	*T*/*C*	*C*	*T*	*T*/*C*
									(*A*) Intravertebral injections									
	12	12	1·0	16	42	2·6	30	233	7·8	45	320	7·1	6	171	28·5	—	—	—
	43	59	1·4	42	70	1·7	75	440	5·9	12	225	18·8	7	971	139·0	—	—	—
	12	12	1·0	21	110	5·2	21	61	2·9	10	26	2·6	25	1506	60·3	—	—	—
	125	125	1·0	25	25	1·0	12	12	1·0	6	240	40·0	17	185	10·9	—	—	—
$\bar{X}$*			1·1			2·2			3·4			11·0			39·8			—
S.E. of mean†			1·1			1·4			1·6			1·8			1·7			—
									(*B*) Intracarotid injections									
	12	12	1·0	12	19	1·6	28	32	1·1	42	170	4·0	20	112	5·6	20	96	4·8
	50	43	0·9	47	60	1·3	117	88	0·8	18	19	1·1	15	62	4·1	12	188	15·7
	12	12	1·0	16	21	1·3	12	52	4·3	12	26	2·2	12	93	7·7	12	48	4·0
	100	125	1·2	25	16	0·6	21	27	1·3	12	25	2·1	12	161	13·4	12	359	29·9
$\bar{X}$*			1·0			1·1			1·5			2·1			6·9			9·8
S.E. of mean†			1·1			1·2			1·4			1·2			1·3			1·6
									(*C*) Intravenous injections									
	12	12	1·0	25	21	0·8	25	12	0·5	25	25	1·0	37	51	1·4	21	110	5·3
	70	50	0·7	30	50	1·7	117	117	1·0	27	25	0·9	25	187	7·5	12	252	21·0
	12	12	1·0	12	16	1·3	12	12	1·0	25	50	2·0	18	57	3·2	11	75	6·8
	90	100	1·1	25	25	1·0	21	23	1·1	10	20	2·0	12	218	18·2	12	563	46·9
$\bar{X}$*			0·9			1·1			0·9			1·4			5·0			13·8
S.E. of mean†			1·1			1·2			1·2			1·2			1·7			1·7

* Antilog $\Sigma \log x_i$. † Antilog standard error of mean of $\log x_i$.

intracarotid injections of 5 μg/kg were ineffective or doubtful. Only once was 10 μg/kg effective and only doses as high as 50 and 100 μg/kg were effective in every case. Intravenous injections produced clear-cut secre-

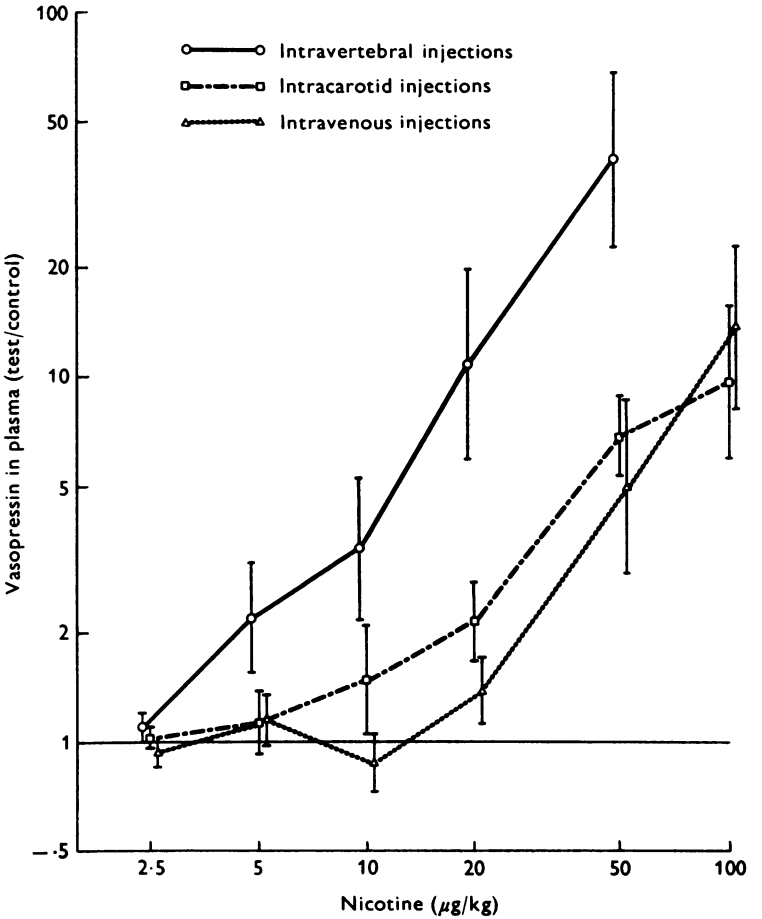

Fig. 1. Vasopressin secretion in response to intravertebral, intracarotid and intravenous injections of nicotine (2·5–100 μg/kg). Vasopressin secretion is expressed as a ratio of plasma concentrations after (*T*) and before (*C*) nicotine injections. Intravertebral injections release vasopressin at a lower threshold and the potency through this route is the highest. No significant differences occur between intracarotid and intravenous injections.

tion in every case only at the level of 100 μg/kg. Analysis of variance of the log transformed data show highly significant differences between routes of administration ($F_{2,53} = 16{\cdot}95$; $P < 0{\cdot}01$), as well as between doses

($F_{4,53} = 20{\cdot}81$; $P < 0{\cdot}01$). The difference between routes is to be entirely ascribed to intravertebral injections because a second analysis of variance between carotid and venous routes showed no significant difference ($F_{1,34} = 2{\cdot}78$; $P > 0{\cdot}05$). Fig. 1, plotted on a log-log scale, shows means and standard errors of means of the log T/C against the log dose, for each of the three routes. The 'vertebral' curve is obviously shifted to the left in a roughly parallel manner, as compared to the 'carotid' and 'venous' curves. This shift to the left suggests a ratio of potency of 4–5 between 'vertebral', on one hand and 'carotid' or 'venous' routes on the other, the latter two being essentially similar.

Effects of nicotine on blood pressure and on heart rate. Fig. 2 shows the effects of intravenous, intracarotid and intravertebral injections of nicotine on mean arterial blood pressure, in doses ranging from 2·5 to 100 μg/kg. Intravenous and intracarotid injections were always followed by a pressor response which appears to be log-dose dependent. It is clear that intravenous injections produce a more intense effect, for every given dose, even though the threshold is similar for both routes. The effects of intravertebral injections on mean arterial blood pressure are more complex: in most experiments (nineteen injections out of twenty-two), low doses of nicotine (2·5–10 μg/kg) produce a dose dependent fall in the mean arterial blood pressure. Higher doses (20 and 50 μg/kg) produce variable results: a depressor response was observed in seven out of twelve injections, whereas a pressor response followed the remaining five injections. This is the cause of the large standard errors of means observed for 20 and 50 μg/kg, in Fig. 2. Fig. 3 shows tracings for intravenous injections: the pressor effects last for 0·5–2 min and the heart rate shows a dose-dependent increase. For higher doses (50 and 100 μg/kg), arrhythmia was usually observed. The pattern of intracarotid responses is similar. Fig. 4 shows typical tracings for intravertebral injections of low doses of nicotine (2·5–10 μg/kg): the depressor effect is typically rapid in its onset, but slow in fading; blood pressure only returns to normal after 10–20 min. The effects of intravertebral injections on heart rate are slight and variable: in most cases, a small drop in frequency is observed.

Effects of intravertebral injections on haemodynamic parameters. Fig. 5 shows the effects of intravertebral injections of nicotine, in doses ranging from 2·5 to 50 μg/kg, given to six cats with a flowprobe implanted around the root of the aorta. The effects on mean arterial blood pressure and on heart rate are similar to those shown in Figs. 2 and 4, indicating that the surgical procedure does not significantly affect the experimental observations. Fig. 5*A* shows that intravertebral nicotine provokes a fall in mean arterial blood pressure, total peripheral resistance and stroke work. This is most pronounced for the lower doses. For higher doses the effects

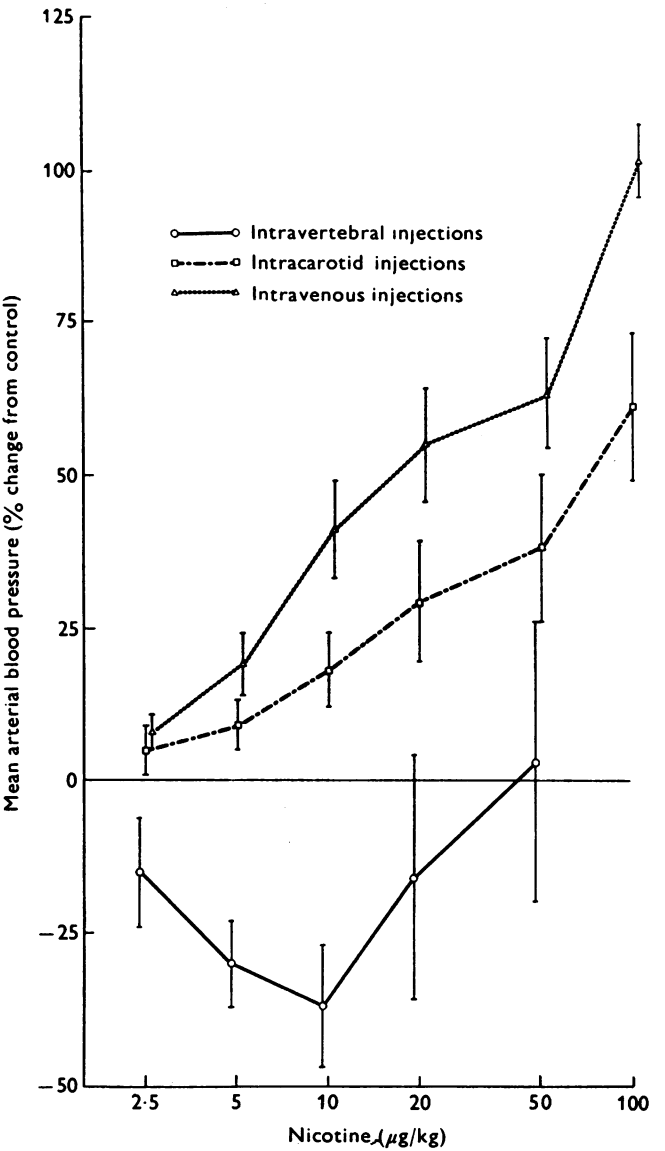

Fig. 2. Effects of intravertebral, intracarotid and intravenous injections of nicotine (2·5–100 μg/kg) on mean arterial blood pressure. Effects are expressed as a percent change from the injection levels. No significant differences were found between such pre-injection levels ($\bar{X}$ = 142·5 mmHg; S.E. of mean = 2·39 mmHg). Intracarotid and intravenous injections produce pressor responses, whereas low doses of intravertebral nicotine produce depressor responses. High doses of intravertebral nicotine produce variable results (see text).

are again variable, which accounts for the large standard errors of means. Aortic flow, heart rate and stroke volume remain essentially unaffected, as shown in Fig. 5*B*. The fall in total peripheral resistance clearly shows an active inhibition of vasomotor tone. The reduction in stroke work can

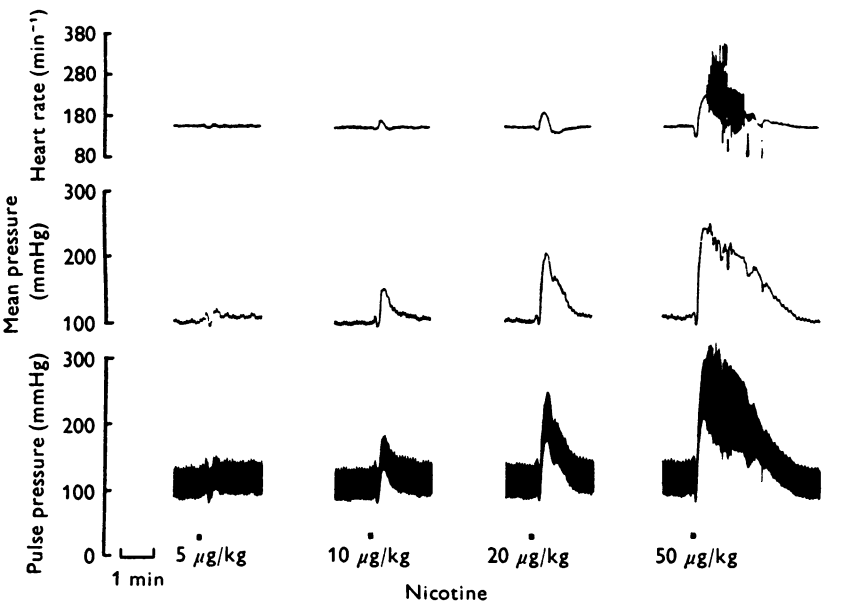

Fig. 3. Effects of intravenous injections of nicotine (5, 10, 20 and 50 μg/kg) on mean and pulse arterial pressure and on heart rate.

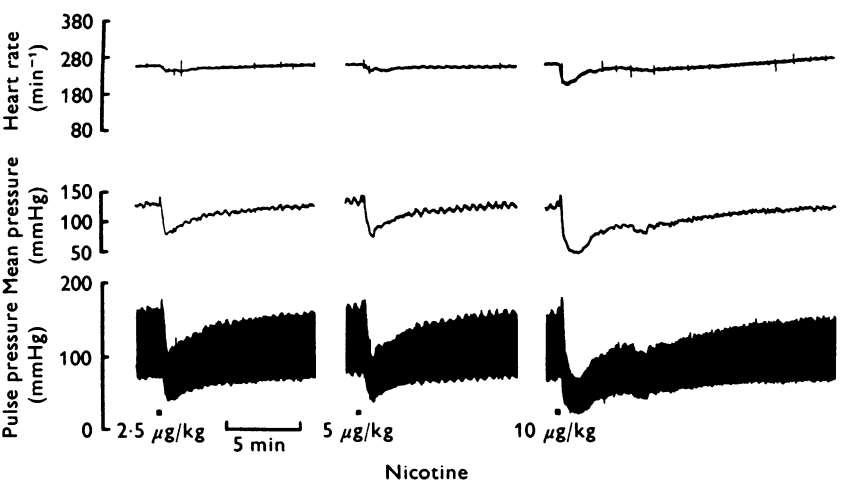

Fig. 4. Effects of intravertebral injections of nicotine (2·5, 5 and 10 μg/kg) on mean and pulse arterial pressure and on the heart rate.

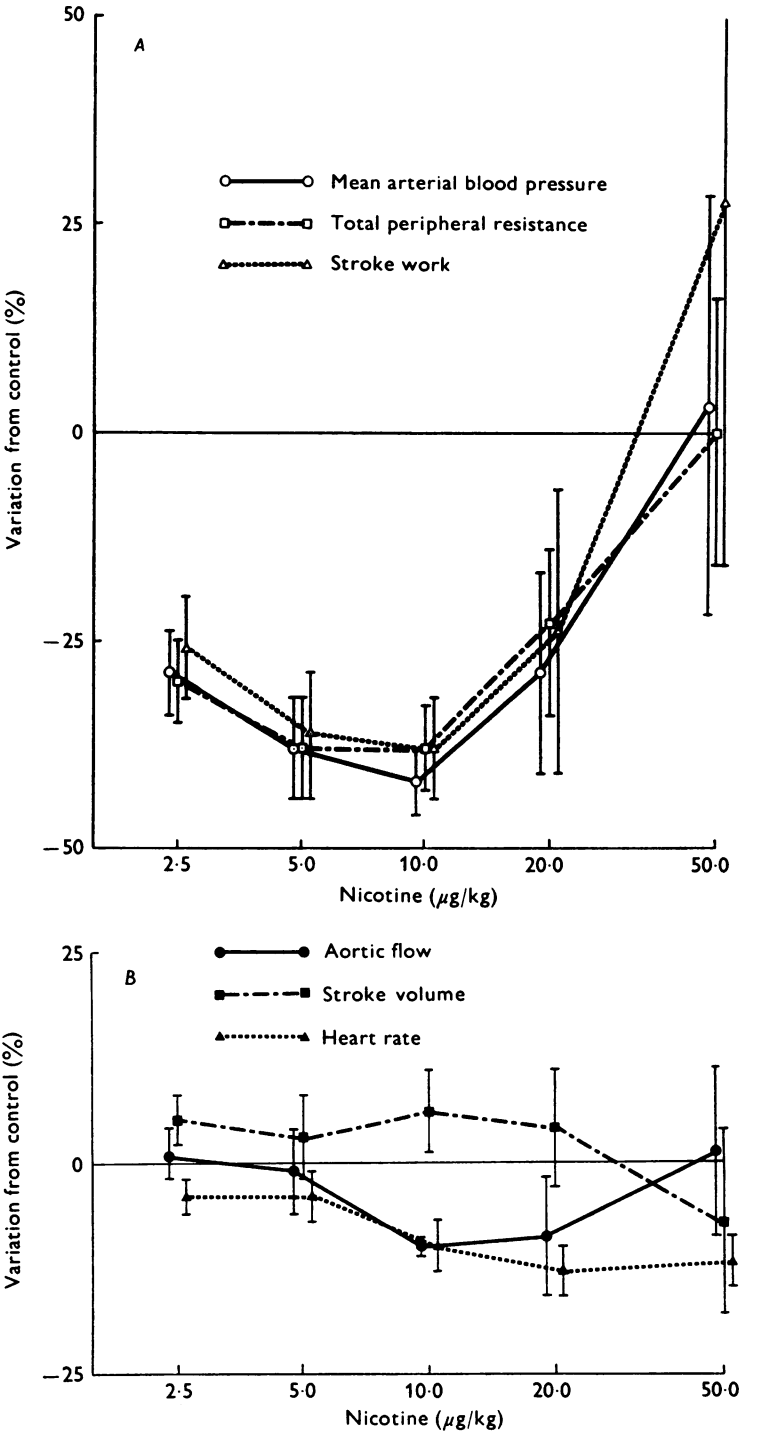

Fig. 5. For legend see facing page.

either be the consequence of a negative inotropic effect or of the reduced afterload, consequent to the vasodilation. Intravertebral nicotine seems to have no effect on chronotropic mechanisms. The overall effects on cardiac function are represented by a constant output and stroke volume in the face of a reduced afterload.

Ear twitch. This phenomenon, described and analysed by Hall & Reit (1966) and by Armitage, Hall, Milton & Morrison (1967) depends on an action of nicotine on the cervical cord, at the level of C1–C2. In our experiments, the ear twitch reflex was consistently observed following intravertebral injections of as little as 2·5 μg/kg nicotine. In every case, the response was restricted to the side of injection. After small doses (2·5 and 5 μg/kg), the response appeared in the form of isolated twitches, roughly 2–3/sec, lasting 20 sec to 3 min. Larger doses (10–50 μg/kg) usually produced an initial sustained laying back of the ear, followed by isolated twitches, the whole process lasting 1–17 min.

Tachyphylaxis. Fig. 6 illustrates the development of tachyphylaxis, following repeated intravertebral injections of nicotine, at 5 or 10 min intervals. It is clear that the depressor effect is progressively abolished. Following complete abolition, the response is restored after 1 hr. The ear twitch response follows a similar pattern. No observations were made on the effect of repeated nicotine injections, at short intervals, on vasopressin secretion.

Dye distribution. The distribution of Cobalt Blue, injected at the end of every experiment was as follows: intravertebral injections produced ipsilateral dying of the cervical cord and brain stem, up to the level of the rostral pons; occasionally the caudal end of the mesencephalon and of the cerebellum were also dyed. In contrast, the distribution space of intracarotid injections of cobalt blue covered the cerebral cortex, diencephalon and in some instances the mesencephalon and the rostral end of the cerebellum. In every case, both after intravertebral and intracarotid injections, Cobalt Blue was entirely restricted to the side of injection.

Fig. 5. Effects of intravertebral injections of nicotine (2·5–50 μg/kg), on cardiovascular parameters of function, expressed as percent change from pre-injections values. In *A*, mean arterial blood pressure, total peripheral resistance and stroke work, all of which are consistently depressed for low doses of nicotine. Higher doses produce variable results. In *B*, aortic flow, stroke volume and heart rate which are not significantly altered. Pre-injection values were as follows: mean arterial blood pressure = $143{\cdot}7 \pm 3{\cdot}8$ mmHg; aortic flow = $102{\cdot}4 \pm 6{\cdot}0$ ml.min^{-1}.kg^{-1}; heart rate = 339 ± 13 min^{-1}; total peripheral resistance = $(50{\cdot}7 \pm 4{\cdot}4)$ 10^3 dyne.cm^{-5}.seg; stroke work = (156 ± 10) 10^3 ergs; stroke volume = $0{\cdot}84 \pm 0{\cdot}06$ ml. (mean $\pm$ S.E. of mean; $n = 30$).

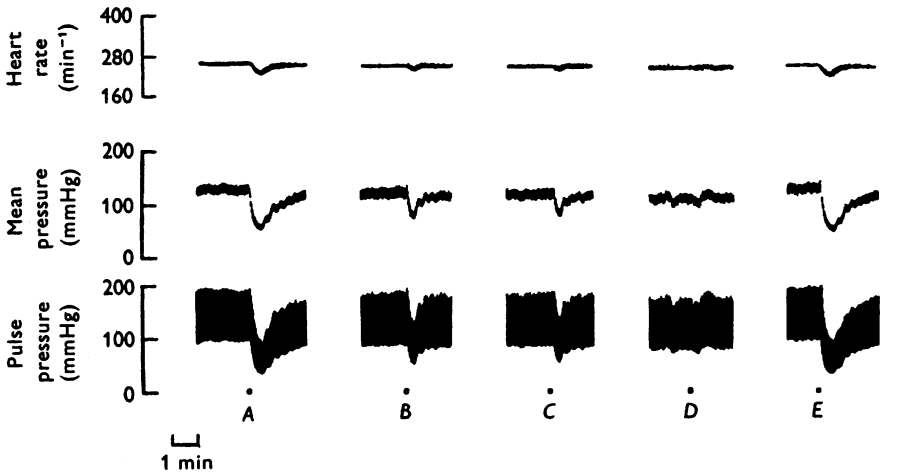

Fig. 6. Tachyphylaxis observed when intravertebral injections of nicotine, 20 μg/kg are repeated at 5 min intervals (*A*, *B*, *C*, *D*). Note the fading of the depressor effect. One hr after the injection marked *D*, a complete recovery of the depressor effect was noted (*E*).

DISCUSSION

The first point to be made about the present results is that the rostral end of the spinal cord and the caudal part of the brain stem can be selectively perfused through the left vertebral artery. The perfusion restricts itself to the side of injection. This allows for the administration of drugs to a limited segment of the central nervous system, without interference with the anatomical and functional integrity of the skull and of the cerebral circulation. It should be noted that only the left vertebral artery allows for such a selective perfusion. Harris & Rocha e Silva (1966) found that the entire brain was bilaterally perfused when cobalt blue was injected into a cannula placed in the right subclavian artery, with its tip opposite the root of the vertebral artery. The problem which arises in this situation is that the root of the right vertebral artery lies very close to that of the right subclavian artery, which in turn arises from the innominate artery, in a position upstream with respect to the two carotid arteries. It is therefore very likely that anything injected through such a cannula will backflow into the innominate and from there into the carotids. When the cannula is placed in the left subclavian, the problem does not arise: the root of the left vertebral artery is far from the root of the subclavian, and this artery in turn arises directly from the aorta in a position which is downstream with regard to the innominate artery.

A number of previously observed effects of topical application of

nicotine to the medulla and upper cervical cord were reproduced by close arterial injection: vasopressin secretion, first demonstrated after topical application of nicotine to the ventral surface of the medulla (Bisset *et al.* 1975); arterial hypotension, due to an action of nicotine in a closely related area (Hall & Reit, 1966; Bisset *et al.* 1975); and the ear twitch reflex, observed when nicotine is allowed to act on the first two segments of the cervical cord (Hall & Reit, 1966; Armitage *et al.* 1967). In addition, a number of original conclusions can be drawn from the present results.

In terms of vasopressin secretion, it should be noted that a dose–effect relationship was established, not only for intravertebral, but also for intracarotid and intravenous injections. These relationships show a potency ratio of 4–5 between the vertebral route, on one hand, and the carotid or venous routes on the other. No significant difference was observed between carotid and venous injections. This result casts doubts over the currently held concept concerning the site of action of nicotine as a stimulus for vasopressin secretion (see Barker *et al.* 1971). It has always been held that systemic administration of nicotine (as, for example, in smoking) releases vasopressin by a direct action on the supraoptic nucleus, which has long been known to possess nicotinic receptors (Duke, Pickford & Watt, 1950). It is certainly true that direct application of nicotine to the region of the supraoptic nucleus is followed by increased rate of firing on the supraopticohypophysial tract (Barker *et al.* 1971), an event which is known to correlate with vasopressin secretion. However, the present experiments show that the potency of intracarotid injections of nicotine is no greater than that of intravenous injections, in sharp contrast to intravertebral injections, 4–5 times more potent. The carotid artery is not, therefore, a close arterial route for vasopressin secretion, even though it irrigates, amongst other structures, the entire hypothalamic area. In this connexion it should be noted that Harris & Rocha e Silva (1966) showed the carotid to be a close arterial route for vasopressin secretion by bradykinin. It appears thus that the ventral surface of the medulla (Bisset *et al.* 1975), rather than any structure within the distribution space of the carotids, is the most sensitive site of action for the vasopressin releasing activity of nicotine. It is thus conceivable that in these experiments, intracarotid injections of nicotine only act after recirculation, and after reaching the ventral surface of the medulla. It should be noted that the concentration of nicotine perfusing the rostral and the caudal ends of the brain may not be equal, depending on the relative rates of flow in the carotid and the vertebral. As concerns the results presented here, this should not prove to be an important issue in that there was no difference between the effects of carotid and venous injections.

In terms of vascular effects, the depressor action of topical application

of nicotine to the ventral surface of the medulla are also confirmed by intravertebral injections, and an analysis of its mechanism of action can be discussed. This depressor effect is long-lasting, only disappearing completely after 15–20 min. When low doses of nicotine (2·5–10 μg/kg) are used, a purely vasodepressor action, accompanied by little or no change in cardiac function, is observed. The only possible explanation for such a result, a fall in resistance accompanied by a fall in transmural pressure is an active reduction in vascular smooth muscle tone in peripheral vascular beds. The reduced stroke work can be explained in terms of the reduced afterload represented by the low peripheral resistance. When higher doses of nicotine are used, slight reductions of cardiac output, stroke volume and heart rate are also observed. For such high doses, the cardiovascular effects are not uniform in different experiments, but an alternation of pressor and depressor effects was noted. The most likely explanation for this lack of uniformity is represented by a masking of the locally induced depressor response by a systemically provoked hypertension. In conclusion, it appears that nicotine acts on medullary neurones mainly concerned with the control of vascular tone; these neurones appear to have a time constant which greatly outlasts the physical presence of nicotine in the region. This fact might have a bearing on the tachyphylaxis, observed when the interval between intravertebral injections is reduced to 10 min or less.

In terms of the ear twitch reflex, no original observations were made. Unilateral responses had been occasionally observed by Hall & Reit (1966); strictly unilateral responses, as described here, were only to be expected within the context of the present experimental design, since the branches which supply the sensitive area for the ear twitch reflex probably arise before the formation of the basilar artery, in the medullary region.

Finally, a word on the interaction of hypotension and vasopressin secretion. Bisset *et al.* (1975) noted that this 'fall in arterial blood pressure cannot be the cause of the vasopressin release produced by the topical application of nicotine', since there was no correlation between the two effects and since vasopressin release could still be obtained after the sinus and vagus nerves were cut (Clark & Rocha e Silva, 1967). The lack of correlation between fall of blood pressure and intensity of vasopressin release was also observed in the present experiments. Moreover, it is clear, as one progresses from low to high doses of intravertebral nicotine, that the depressor effect is replaced, in many experiments, by a pressor effect, with no perceptible change in the slope of the log dose $\times$ log T/C for plasma vasopressin.

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